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1: Nat Biotechnol. 2003 May;21(5):526-31. Epub 2003 Apr 14. Related Articles, Links

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nature biotechnology

Complete genome sequence and comparative analysis of the industrial microorganism Streptomyces avermitilis.

Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S.

Kitasato Institute for Life Sciences, Kitasato University, Kanagawa 228-8555, Japan.

Species of the genus Streptomyces are of major pharmaceutical interest because they synthesize a variety of bioactive secondary metabolites. We have determined the complete nucleotide sequence of the linear chromosome of Streptomyces avermitilis. S. avermitilis produces avermectins, a group of antiparasitic agents used in human and veterinary medicine. The genome contains 9,025,608 bases (average GC content, 70.7%) and encodes at least 7,574 potential open reading frames (ORFs). Thirty-five percent of the ORFs (2,664) constitute 721 paralogous families. Thirty gene clusters related to secondary metabolite biosynthesis were identified, corresponding to 6.6% of the genome. Comparison with Streptomyces coelicolor A3(2) revealed that an internal 6.5-Mb region in the S. avermitilis genome was highly conserved with respect to gene order and content, and contained all known essential genes but showed perfectly asymmetric structure at the oriC center. In contrast, the terminal regions were not conserved and preferentially contained nonessential genes.

Publication Types:
- Evaluation Studies
- Validation Studies

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〔特許手続上の微生物の寄託の国際的承認に関するブダペスト条約〕

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

下記国際寄託当局によって規則7．1に従い発行される

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

原寄託についての受託証

issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page.

寄託者

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I．微生物の表示	
（寄託者が付した識別のための表示） *Streptomyces avermitilis* K2038	（受託番号） 微工研条寄第　2775　号 （FERM BP- 2775　）

II．科学的性質及び分類学上の位置

I欄の微生物には、次の事項を記載した文書が添付されていた。

☒ 科学的性質

☒ 分類学上の位置

III．受領及び受託

本国際寄託当局は、平成　2　年　2　月　26　日（原寄託日）に受領したI欄の微生物を受託する。

IV．国際寄託当局

通商産業省工業技術院微生物工業技術研究所

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2

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

J. Sambrook
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

E.F. Fritsch
GENETICS INSTITUTE

T. Maniatis
HARVARD UNIVERSITY

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Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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exonuclease activity, is superior to the fragment produced by subtilisin treatment of *E. coli* DNA polymerase I.

The most common types of systematic sequencing problems are listed in Table 13.5 together with suggestions for solving them. When problems of this type arise, it is often useful to use the reagents provided in commercial sequencing kits to identify the defective component of the chain-extension/chain-termination reaction.

Problems with Polyacrylamide Gels

Problems with polyacrylamide gels are of two types: (1) technical difficulties arising from the use of poor reagents (see Table 13.5) and, more rarely, (2) problems caused by secondary structure within the template or radiolabeled product. Problems due to secondary structure appear in two forms:

- *Blocks* caused by regions of secondary structure (e.g., homopolymeric tracts) in the template strand that severely impede the progress of the DNA polymerase. These blocks can sometimes be relieved by using reverse transcriptase, *Taq* DNA polymerase, or Sequenase instead of the Klenow fragment of *E. coli* DNA polymerase I or by performing the sequencing reactions at 55°C (Gomer and Firtel 1985). In a few cases, however, the blocks and the sequences that lie beyond them can be sequenced only by the Maxam-Gilbert method.
- *Compressions*, in which bands in a particular location within the gel are crowded together, resulting in unreadable sequence. In the region of the gel above the compression, the space between the bands frequently becomes abnormally large. These are gel artifacts caused by short stretches of dyad symmetry—especially those containing a high proportion of G and C residues—at the 3′ terminus of the radiolabeled strand. These regions of ambiguity can often be resolved by sequencing the opposite strand. If the problem persists, try substituting the base analog dITP for dGTP in the chain-extension/chain-termination reaction. This analog forms I-C base pairs that contain only two hydrogen bonds instead of the three normally formed by G-C base pairs. Although the Klenow fragment of *E. coli* DNA polymerase I will accept dITP, Sequenases incorporate the analog more efficiently and are therefore the enzymes of choice when carrying out sequencing reactions with dITP. Because the use of dITP accentuates pauses in the chain-extension/chain-termination reaction, sequencing reactions containing the base analog should always be run in parallel with reactions containing dGTP. The concentrations of dNTPs and ddNTPs in the stock labeling mixture and chain-extension/chain-termination mixtures when dITP is substituted for dGTP are given below.

  dITP stock labeling mixture

dITP (0.5 mM)	30 µl
dCTP (0.5 mM)	15 µl
dTTP (0.5 mM)	15 µl
deionized H_2O to a final volume of 1 ml	

Dispense the dITP stock labeling mixture into aliquots in microfuge tubes and store them at −20°C.

ddITP chain-extension/chain-termination mixtures

Dilute stock solutions of ddNTPs (10 mM) (see page 13.44) with water to a final concentration of 0.5 mM. Use the diluted stock solutions of ddNTPs and the 0.5 mM stock solutions of dNTPs (see page 13.44) to make up chain-extension/chain-termination mixtures as shown below. Dispense the ddITP chain-extension/chain-termination mixtures into aliquots in microfuge tubes and store them at −20°C.

ddNTP mixture	0.5 mM Stock solutions (μl)				Diluted working solution of ddNTP[a] (0.5 mM)(μl)	5 M NaCl (μl)	H_2O (μl)
	dITP	dATP	dTTP	dCTP			
ddI	320	160	160	160	3	10	187
ddA	320	160	160	160	16	10	174
ddT	320	160	160	160	16	10	174
ddC	320	160	160	160	16	10	174

[a] Use the appropriate ddNTP for the mixture being made; for example, for the ddI mixture, use 3 μl of the diluted working solution of ddITP (0.5 mM).

The quality of sequence obtained is never as good when base analogs are used instead of conventional dNTPs. It is therefore helpful to load each set of four sequencing reactions into eight adjacent lanes of a polyacrylamide gel in the order IATCITAC. This ensures that each of the four sequencing reactions is adjacent to the other three and allows the order of closely spaced bands to be determined more easily.

Compressions not resolved by dITP can occasionally be resolved by using 7-deaza-dGTP (Mizusawa et al. 1986). However, dITP is usually the more effective base analog and is therefore the first choice for resolving compressions. If problems persist, the addition to the chain-extension/chain-termination reactions of 0.5 μg of single-stranded DNA-binding protein from *E. coli* usually eliminates the difficulty. When using single-stranded DNA-binding protein (United States Biochemical 70032), it is necessary to treat the reactions with proteinase K (0.1 μg/reaction) for 20 minutes at 65°C after adding the formamide/EDTA/XC/BPB gel-loading buffer. This allows the DNA to enter the sequencing gel and prevents smearing of bands.

The substitution of dITP for dGTP causes nucleic acids to migrate slightly faster through denaturing polyacrylamide gels.